Fungus-Induced Biochemical Changes in Peanut Plants and Their Effect on Development of Beet Armyworm, *Spodoptera Exigua* Hübner (Lepidoptera: Noctuidae) Larvae

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ABSTRACT In choice tests, beet armyworm (BAW), *Spodoptera exigua*, larvae feed preferentially on leaves from peanut plants, *Arachis hypogaea* L., previously infected by the white mold fungus, *Sclerotium rolfsii* Saccodes (mitosporic fungi) (Cardoza et al. 2002). In this study we determined that third instar BAW caterpillars allowed to feed on *S. rolfsii*-infected plants had significantly higher survival, produced significantly heavier pupae, and had shorter time to pupation than those allowed to feed on healthy plants. Leaf tissue from white mold infected peanut plants contained similar levels of soluble and insoluble protein, but significantly higher levels of soluble sugars. In addition, white mold-infected plants had significantly lower starch content and total soluble phenolics compared with leaves from healthy plants. Levels of jasmonic acid were similar in plants attacked by either the fungus or BAW, but were significantly higher in plants that were infected by the fungus and then fed on by BAW. Salicylic acid (SA) levels in fungus-infected plants were not significantly different from those of control plants. However, levels of SA in plants damaged by BAW alone were significantly lower than those of plants under simultaneous attack by the fungus and BAW.

KEY WORDS *Spodoptera exigua*, plant-insect interaction, plant biochemistry, white mold, beet armyworm, larval development

HERBIVOROUS INSECTS AND PLANT diseases are serious problems in agriculture because they reduce yield and quality of crops. The study of physiological and chemical changes induced in plants by the attack of insects and pathogens has independently captured the interest of scientists in the areas of plant pathology and entomology. The primary focus of the resulting studies has been to develop potential plant immunization methods as a means to prevent pest outbreaks (Yedidia et al. 1999, Lucas 1999). Systemic acquired resistance (SAR) is a nonspecific resistance induced throughout the plant by local infection with a variety of pathogens (Kuc 1982, Dean and Kuc 1985). Recent studies have provided evidence for the key role of salicylic acid (SA) in inducing SAR (Gaffney et al. 1993; Delaney et al. 1994, 1995). Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) is a synthetic SA analog capable of inducing SAR. BTH also induces disease resistance in a number of plant species and this SAR is not because of an accumulation of SA (Friedrich et al. 1996).

While previous exposure to a pathogen can provide plant resistance to future attacks by the same pathogen species, the induced changes can also have interspecific consequences. Different plant defense pathways are generally induced in response to insect herbivores and pathogen attack. Jasmonic acid (JA) induces expression of defense compounds such as proteinase inhibitors and polyphenol oxidases, which provide plant resistance against insects (Ryan 1990, Farmer and Ryan, 1990, Farmer et al. 1992, Thaler et al. 1996, 2001). Salicylic acid (SA), however, is involved in the production of pathogenesis-related proteins and phytoalexins in response to pathogens (Delaney et al. 1994, Narusaka et al. 1999, Murphy et al. 2000). Another mediator of plant defense responses is the highly volatile plant hormone, ethylene. Expression of wound- and JA- inducible plant responses has been shown to be regulated by ethylene or SA (Fidantsef et al. 1999, O'Donnell 1996, Stout et al. 1999, Van Loon 1997).

Pathogen infection can alter host plant biochemistry in such a way that the feeding preference and performance of phytophagous arthropods may be positively or negatively affected. For example, Karban et al. (1987) reported that the development of spider mites, *Tetranychus urticae*, was negatively affected when they fed on cotton plants that had been previ-

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ously inoculated with the fungus *Verticillium dahliae.* In contrast, beet armyworm (BAW), *Spodoptera exigua*, larvae preferentially fed and consumed larger quantities of leaves from white mold, *Sclerotium rolfsii*, infected plants (Cardoza et al. 2002). Stout at al. (1999) and Thaler et al. (1999) found that tomato plants that had been previously treated with BTH, were fed on to a greater extent by BAW and corn earworm, *Helicoverpa zea*, than untreated controls. In a study of proteinase inhibitor gene expression, tomato plants treated with BTH were determined to have compromised direct defenses against herbivores (Thaler et al. 1999, Fidantsef et al. 1999). Thus, the finding that BAW preferred leaves from white mold infected peanut plants (Cardoza et al. 2002) could have been caused by compromised direct defenses of the plant because of fungal infection. However, pathogens have been found to affect the levels of compounds such as sugars, starch, and proteins in their host plants (reviewed in Hatcher 1995). Major nutrients such as carbohydrates and proteins are listed as some of the most important phagostimulants for phytophagous insects (Matsuda 1988); whereas, secondary plant defensive compounds such as tannins (phenolic compounds) and protease inhibitors can have the opposite effect (Slansky and Scriber 1985). Therefore, it is possible that the feeding preference of BAW for leaves from fungus-infected plants could have been influenced by changes in secondary metabolite content and/or the nutritional quality of the plant tissues.

In the current study, we examine the effect of *S. rolfsii* infection on the levels of primary (nutrients) and secondary (defense substances) metabolites, and on the levels of plant defense signaling molecules in peanut plants. Experiments were also conducted to evaluate the performance of BAW larvae, from third instar to pupation, on either healthy or white mold infected peanut plants. The levels of nutrients, such as sugars, starch, and proteins, and of secondary defense substances such as soluble phenolics, and proteinase inhibitors in leaves of healthy and infected plants were compared. The latter analyses were conducted to determine a possible correlation between the levels of these compounds and the performance of the insects on these plants. Levels of the defense signaling molecules jasmonic acid (JA) and SA in plants under individual and simultaneous pathogen and BAW attack were also determined.

Materials and Methods

Plant and Insect Material. 'Georgia Green' peanut, *Arachis hypogeae*, seeds were sown in pairs in 3.78-liter pots (16 cm diameter) containing a 1:1 (vol:vol) mixture of commercially available filter sand and Metromix 300 (Scotts-Sierra Horticultural Company, Marysville, OH). Plants were grown in an insect-free greenhouse with natural light, under Florida summer conditions (14 light:10 dark light cycle). The greenhouse temperature was kept between 25 to 30°C. After emergence, seedlings were thinned to one individual per pot. Each plant received 100 ml of a 3.38 g/liter liquid fertilizer solution (20-20-20 [N-P-K] Peters, W. R. Grace, Fogelsville, PA) every 2 weeks starting 1 week after emergence. At the beginning of all experiments, plants were 5-weeks old and had six fully developed leaves on the main stem and three fully developed leaves on each of two secondary branches. Beet armyworm eggs were obtained from the rearing facilities at the USDA-IBPMRL, Tifton, GA. Larvae were reared on a pinto bean-based artificial diet following the methodology described by King and Leppla (1984). Insects were kept in a biological incubator with a 14:10 L:D cycle maintained at 25°C. Newly molted third instar larvae were used in all experiments.

Fungal Culture. *S. rolfsii* (strain 80) was grown on potato dextrose agar (PDA) petri plates. Fresh cultures were started, under sterile conditions, by placing sclerotia harvested from the original culture in the center of PDA media plates. Culture plates were kept in a biological incubator with a 14:10 L:D cycle and maintained at 25°C and 60 to 70% RH° for 3 d. To inoculate the experimental plants, fungal culture plugs (5 mm diameter) were cut out of the agar plate with a #2 cork borer.

***S. rolfsii* Infection on Peanuts.** Peanut plants were infected with *S. rolfsii* by distributing four culture plugs along the main stem. The plugs were pressed against the stem so they remained in place and so the fungus was in direct contact with the stem. Each plant was then individually covered with a 3.78-liter plastic storage bag (Ziploc DowBrands L. P., Indianapolis, IN) to provide adequate humidity and temperature conditions for fungal growth and colonization of the plant's stem. Noninoculated control plants were similarly covered with the plastic bags. The plants were incubated for 3 d in the greenhouse. After this period, lesions of ≈1 cm long could be observed at the point of fungal contact with the stem. Bags were removed from the plants 24 h before being used for the experiment. *S. rolfsii* is a nonsystemic pathogen and only the stems of the plants were in contact with the fungus; therefore, the leaves that were consumed by the caterpillars were not directly infected by the fungus.

BAW Performance on Healthy and Fungus-Infected Peanut. Healthy and fungus-infected plants were individually placed within 46 × 46 × 46 cm Plexiglas cages into which six third instar BAW were introduced. Although the insects were not weighed at the time of introduction, care was taken so that all larvae used were newly molted third instars. Cages were kept in the greenhouse throughout the experiment. One plant provided more than enough food for all insects to develop until pupation. Insects were observed daily and were removed from the plants when they reached the wandering stage and no more feeding activity was observed. All insects from each of the treatments were collectively placed into a petri plate carefully labeled to indicate plant treatment and replicate number and kept in an incubator, under the conditions described above, until pupation. At that time, the time of pupation, number of surviving pupae

and their weights were recorded. Four replicates of this experiment were set up at one time in the greenhouse and the experiment was repeated two more times to obtain a total of 12 replicates.

Plant Samples. Plant samples consisted of eight tetrafoliate leaves removed from the main-stem of plants that were treated as follows: (1) healthy (no fungal infection, no insect damage), (2) exposed to feeding by six BAW for 24 h, (3) fungus infected 4 d before harvesting, (4) fungus-infected 4 d before and exposed to feeding by six BAW for 24 h before tissue was harvested. After removing the insects from the plants, all the leaves of six plants from each of the above treatments were finely ground in liquid N_2 with a mortar and pestle immediately after removal from the plant. All leaf samples were kept in a −70°C freezer until needed for the analyses.

Carbohydrates Analysis. Five hundred mg (fresh weight) of the leaf samples were combined with 2 ml of 80% acetone and then incubated in a 75°C water bath for 10 min. Then, the samples were centrifuged at 5000 × *g* for 10 min, the supernatants were transferred to new tubes and the pellets were re-extracted and centrifugation was repeated. The supernatants were combined and then evaporated to dryness in a Savant SC210A speed-vac (Savant Instruments, Inc. Holbrook, NY). The residue was resuspended in 1 ml of purified H_2O and this was used to estimate total soluble sugars. Total soluble sugars and glucose from starch digestion were determined by the phenol-sulfuric acid method that uses commercially available glucose as a standard and measures absorbency at 490 (Dubois et al. 1956). The remaining insoluble residue was dried overnight in a 70°C oven. The following day, the residues were gelled by adding 0.20 ml 95% ethanol and 4 ml of 50 mM acetate buffer (pH = 4.5). The gelled residues were placed in boiling water bath for 15 min and then cooled by placing in an ice bath before adding 0.1 ml of heat stable α-amylase solution (68300 U/ml) (Sigma Co., St. Louis MO). Samples were incubated overnight at 50°C and then centrifuged at 5000 × *g* for 12 min. The supernatant was then used to estimate the amount of glucose cleaved from starch using the phenol-sulfuric acid method.

Protein Analysis. For protein quantification, 50 mg of each of the leaf samples were homogenized in 500 μl of chilled buffer-A, consisting of 0.05 M $NaPO^4$ at pH 7.5 (Sigma, St. Louis, MO, USA). The homogenate was centrifuged at 14000 × *g* for 15 min and the supernatant containing soluble proteins was transferred to a new micro-centrifuge tube. The pellet was washed and centrifuged, as above, three times using 1 ml of buffer-A each time and discarding the supernatant. The pellet was resuspended by vortex mixing in 500 μl of buffer-B, consisting of 62.5 mM Tris-HCl pH 6.8, 2% SDS (w:v) and 10% glycerol (v:v). Following centrifugation at 14,000 × *g* for 15 min, the supernatant containing the buffer-insoluble proteins was transferred to a new microcentrifuge tube. Protein quantity was determined with the Bio-Rad DC Protein Assay (Alam 1992) using bovine serum albumin fraction V as a standard.

Proteinase Inhibitors Analysis. Trypsin proteinase inhibitor activity was determined following the method of Hummel (1958), where *N*-α-benzoyl-DL-arginine p-nitroanilide (BAPNA) is used as a colorimetric substrate. Amount of trypsin proteinase inhibitors is expressed in μg/g fresh weight estimated using commercially available p-nitroaniline (Sigma Co., St. Louis, MO) as a standard. Cysteine proteinase inhibitor activity was determined using the papain assay based on Koiwa et al. (1998). In this case, N-α-benzoyl-DL-arginine-β-naphthylamide (BANA) was used as the colorimetric substrate. In both cases absorbance was read with a spectrophotometer set at 430 nm for the trypsin and at 540 nm for the cysteine proteinase inhibitors. Cysteine inhibitory activity is expressed as the percentage decrease in absorbance relative to the reaction with no plant extract.

Soluble Phenolic Analysis. For extraction of soluble phenolics, 500 mg of the samples were extracted with 10 ml of 1% HCl in methanol, vortexed for 20 min and then centrifuged at 1000 × *g* for 10 min. The amount of total soluble phenolics in each of the samples was determined using the Folin-Denis method (Swaine and Goldstein 1964). This procedure determines total free phenolics based on the reduction of phosphomolybdic acid by phenols. Absorbance was read in a spectrophotometer (Spectro 22; Labomed, Culver City, CA) set at 760 nm wavelength. Commercially available tannic acid (Sigma Co.) was used as a standard.

Jasmonic Acid and Salicylic Acid Analysis. The extraction and quantification of JA was modified from Weber et al. (1997). Leaf tissue samples of ≈1 g were extracted in 3.5 ml MeOH with 500 ng of the internal standard dihydrojasmonic acid (dhJA). After 30 min in a sonicating bath, each sample was mixed with 1.5 ml of purified H_2O, and centrifuged at 3000 × *g* for 5 min. The resulting supernatant was saved, adjusted to pH 8.5 with aqueous 1 M NH_4OH and kept on ice. Solid phase extraction (SPE) cartridges (Reverse Phase C18, 12 ml, Mallinckrodt Baker, Griesheim, Germany) were washed with 8 ml each of 100% MeOH, followed by 70% MeOH. Each sample was passed through the SPE cartridge followed by 7 ml of 75% MeOH. All eluate was collected, adjusted to pH 3.5 with 10% formic acid, and the volume was raised to a total of 50 ml with H_2O. The SPE cartridges were cleaned and conditioned for reuse with 5 ml each of 0.8% formic acid in MeOH, 100% MeOH, diethylether, 100% MeOH, and finally 10 ml H_2O. The samples were then reloaded on the cartridges and washed with 7 ml each of 85:15 H_2O:EtOH and H_2O. After all water was removed from the columns, the oxylipin fraction was eluted with 10 ml diethylether, and the eluate was transferred to a 2 ml reaction vial where it was dried under N_2. Methanolysis was performed by incubating with 30 μl of a 1:2 (v:v) HCl:MeOH mixture for 12 h at 30°C. The HCl:MeOH was then completely removed under a stream of N_2 gas and each sample was brought up to 75 μl in $MeCl_2$. Samples were analyzed by gas chromatography-mass spectrometry (GC-MS) on a Hewlett–Packard (HP) 6890 GC (He carrier gas;

0.7 ml min^{-1}; splitless injector 240°C, injection volume 2 μl) with a HP-5MS column (5% phenyl methyl siloxane, 30 m × 250 95 m i.d. × 0.25 95 m film thickness) with the temperature programmed from 40°C (1 min hold) at 10°C min^{-1} to 240°C (hold for 15 min). The GC was coupled to a HP 5973 quadrapole-type mass selective detector with transfer line, source, and quadrapole temperatures of 230, 230, and 150°C, respectively.

SA levels were measured following a procedure by Uknes et al. (1993). Ground plant tissue (0.5 g) was first extracted with 3 ml of 90% methanol, then re-extracted with 2 ml 100% methanol. The extracts were combined, divided into two equal parts, and carefully dried down under vacuum. For free SA determination, samples were resuspended with 2.5 ml 5% trichloracetic acid (TCA). For the estimation of conjugated SA, samples were brought up in 1 ml of water, acidified to a pH of one with HCl and boiled for 30 min to cleave the glycoside conjugates. Separate free and conjugated SA samples were each extracted with 2.5 ml ethyl acetate: cyclopentane: isopropanol (100:99:1), dried down and then resuspended in 20% methanol. The amount of SA in each of the samples was determined by reverse phase high performance liquid chromatography on a 4.6 × 250 mm 5 μm C-18 column (Beckman Ultrasphere, Fullerton, CA) with a Waters 474 scanning fluorescence detector (excitation energy 295 nm, emission energy of 400 nm). Data presented is on the total (free + conjugated) SA.

Statistical Analysis. The effect of trial (block) was not significant. Therefore, data for performance of BAW on healthy and fungus-infected peanut were analyzed by *t*-test (Proc MEANS, SAS Institute, 1996). Data on nutritional analysis and on levels of JA and SA of peanut leaves were analyzed using analysis of variance (ANOVA) (Proc GLM, SAS Institute 1996). The interaction between the different treatment factors (BAW*Fungus) on the levels of JA and SA was not significant. Only significant ANOVAs were followed by Tukey's mean separation test.

Results

BAW Performance on Healthy and Fungus-Infected Peanut. In a no-choice situation, BAW larvae reared on fungus-infected peanut plants had significantly higher survival ($t = 3.37$; df = 53; $P = 0.0014$) (Fig. 1A), developed significantly faster, based on number of days from third instar to pupation ($t = 29.9$; df = 53; $P = 0.0001$) (Fig. 1B), and produced significantly heavier pupae ($t = 2.96$; df = 53; $P = 0.0045$) (Fig. 1C) than on their healthy counterparts. Overall, insects reared on fungus-infected peanut plants had ≈20% higher survival rate, developed more than 4 d faster and were 25% heavier than those reared on healthy plants.

Plant Nutritional Analysis. Plants infected by the fungus had significantly higher soluble sugar content ($F = 10.6$; df = 3, 36; $P < 0.0001$) and significantly lower starch content ($F = 4.8$; df = 3, 36; $P < 0.0064$) than healthy plants and plants damaged by BAW alone (Table 1). Soluble and insoluble protein, and trypsin proteinase inhibitor activity were not significantly different among the treatments tested. However, cysteine proteinase inhibitor activity was higher in plants attacked simultaneously by the fungus and BAW ($F = 2.68$; df = 4, 156; $P < 0.0489$) compared with undamaged control plants or in those that were fungus-infected or damaged by BAW alone (Table 1). Additionally, the concentration of soluble phenolics was significantly lower in plants infected with fungus ($F = 3.5$; df = 4, 35; $P < 0.0163$).

JA and SA Levels. Plants that were infected with the white mold fungus or damaged by BAW had similar levels of JA (Table 1). However, plants that were infected by the fungus and then exposed to BAW had the highest detectable levels of JA and these were significantly different from those in all of the other treatments ($F = 2.53$; *df = 4, 35;* $P < 0.0001$) (Table 1). JA was not detected in undamaged/uninfected control plants (Table 1). However, the levels of SA in plants damaged by the insects alone were significantly lower than those in the fungus/BAW treated plants ($F = 3.25$; df = 4, 35; $P < 0.001$) (Table 1). Levels of salicylic acid in plants under the other treatments did not differ significantly from each other (Table 1).

Discussion

BAW have been reported to prefer feeding on leaves from white mold-infected over those from healthy peanut plants (Cardoza et al. 2002). In the current study, we found that these insects survived better, developed faster, and reached a greater pupal weight when reared on white mold-infected peanut plants. Following nutritional analysis of leaf tissue, we found that leaves from fungus-infected plants had higher levels of nutrients (sugars) and lower levels of defensive substances (soluble phenolics) than their healthy counterparts, which may have contributed to the observed BAW feeding preference and positive effect on their performance. Some species of grasshoppers prefer feeding and perform better on sunflower leaves infected by a rust fungus (Lewis 1979, 1984). Sucrose has been found to be a strong phagostimulant for grasshoppers and other insects (Cook 1977, Dethier 1982, Bernays and Weiss 1996); thus, BAW feeding preference and enhanced performance may have been a result of the higher concentration of soluble sugars we found in the tissue from white mold infected plants. Plant phenolics are induced by herbivore feeding on plants (Einhellig 1999) and may reduce food digestion by insects because of their astringent properties (Levin 1971). These compounds can form complexes with proteins in general; thus, they may interfere with absorption of plant protein and may affect the activity of herbivore digestive enzymes (Levin 1971, 1976, Felton et al. 1989, 1992, Haggerman and Butler 1991). Phenolic compounds have also been reported as being induced in plants by fungal infection (Levin 1976, Hammerschmidt and Nicholson 1999) and they have been found to confer resistance to plants against certain fungal pathogens

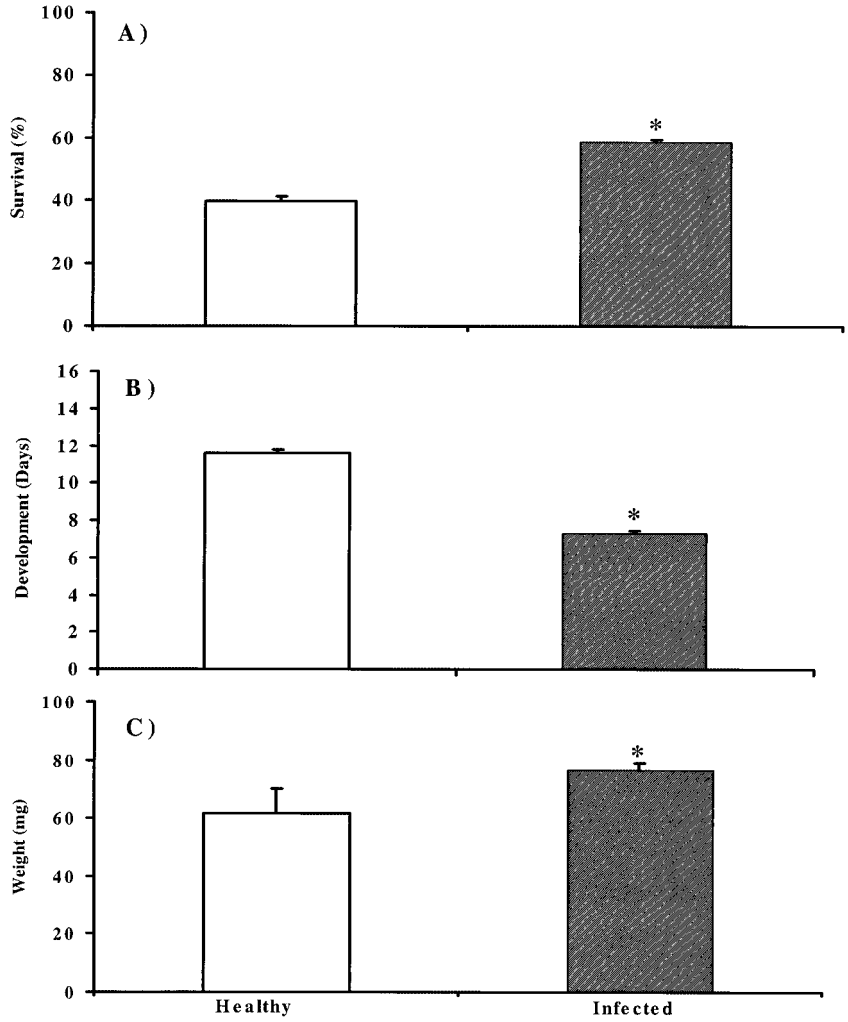

Fig. 1. BAW larval performance, from third instar to pupae, on healthy and white mold-infected peanut in a no-choice situation: A) mean percent survival, B) mean developmental time (days) from third instar to pupae, and C) mean pupal weight (mg). Light bars represent healthy control plants and dark bars represent white mold infected plants. Error bars denote one SE

(Walker and Stahmann 1955). Thus, it was surprising to find that the level of soluble phenolics in plants infected by the white mold in our study was actually lower than that in control plants and in plants damaged by BAW alone.

Trypsin-like proteinases are the major active digestive proteinases in the guts of *Spodoptera* larvae (Broadway 1995). Plant proteinase inhibitors, especially in the serine class, such as trypsin and chymotrypsin inhibitors, have been reported to slow larval development in BAW and other lepidopteran larvae (Broadway and Duffy 1986, Broadway et al. 1986). The development of BAW was significantly retarded with the addition of as little as 0.045% potato chymotrypsin II and 0.09% w. wt. soybean trypsin inhibitor to a casein-based diet (Broadway and Duffy 1986). Plant proteinase inhibitors have also been found to negatively affect the growth of fungal pathogens (Lorito et al. 1994). Recently, a trypsin-like proteinase inhibitor with antibiotic properties against the fungus *Sclerotinia sclerotiorum* has been isolated from sunflowers (Giudici 2000). In our study, the level of trypsin-like proteinase inhibitors did not differ among the different treatments, but the level of cysteine proteinase inhibitor activity was significantly higher in plants infected by the white mold fungus and damaged by BAW. Recent studies suggest that cysteine proteinase inhibitors have a very active role in the defense of plants against pathogens (Giri et al. 1998, Joshi et al. 1999).

The effect of fungal infection on insect feeding preference and their performance on plants vary widely. For example, Kluth et al. (2001) found that when given a choice between healthy and rust-in-

Table 1. Mean (SE) amounts of selected nutrients and hormones in leaves of healthy (Control), BAW-damaged (BAW), white mold-infected (Fungus), and white mold-infected + BAW-damaged (Fungus + BAW) peanut plants

Compound/Treatment[a]	Control	BAW	Fungus	Fungus + BAW
Soluble protein[b]	20.3 (1.4)a	20.2 (5.0)a	23.2 (5.5)a	22.5 (2.5)a
Insoluble protein[b]	10.0 (1.0)a	8.0 (0.4)a	8.0 (0.5)a	8.4 (0.8)a
Soluble sugars[c]	99.5 (6.0)b	81.2 (3.7)a	120.3 (5.0)c	107.25 (5.1)bc
Starch[c]	117.1 (5.0)a	111.3 (8.5)a	89.2 (4.5)b	83.6 (4.1)b
Soluble phenolics[c]	159.2 (4.7)a	154.3 (4.7)a	109.6 (9.2)b	108.4 (9.8)b
Trypsin proteinase Inhibitor[c]	12.4 (0.3)a	11.8 (0.2)a	11.8 (0.2)a	12.0 (0.4)a
Cysteine proteinase inhibitor[d]	60.1 (1.9)a	58.5 (4.6)a	63.8 (1.5)a	71.5 (1.8)b
Jasmonic acid[e]	ND	25.5 (1.4)a	16.0 (2.5)a	73.6 (10.6)b
Salicylic acid[e]	1222 (108)ab	798 (69.4)a	1024 (73.8)ab	1539 (122)b

ND = not detected.
[a] Means within rows followed by the same letter are not statistically different (Tukey's mean separation test, $P = 0.005$).
[b] Amount in mg/g fresh weight.
[c] Amount of compounds expressed as μg/g of fresh weight.
[d] Percent decrease in absorption relative to control reaction without plant extract.
[e] Amount expressed in ng/g of fresh weight.

fected thistle, chrysomelid beetles preferred to feed on the healthy plants. Also, Hatcher et al. (1995) found that infection of *Rumex* spp. by rust fungi increased feeding preference by the chrysomelid beetle *Gastrophysa viridula*, but larval performance and adult reproductive capacity were negatively affected. In the same study, rust-infected leaves were found to have lower nitrogen and higher oxalate concentration than healthy leaves, which might account for the negative effects observed in the insects. Moran (1998) found that discs from cucumber, *Cucumis sativus*, leaves infected with *Cladosporium* fungi were preferably fed on by cucumber beetles, *Diabrotica undecimpunctata howardii*. Similarly, other studies have found that plant defensive responses against pathogens have a negative effect on the plant's ability to cope with insect herbivory (Karban et al. 1987, Bostock 1999, Felton et al. 1999, Fidantsef et al. 1999, Stout et al. 1999). However, there have also been reports suggesting enhanced plant defenses against insects in plants after pathogen inoculation. For example, McIntyre et al. (1981) found that infection of tobacco, *Nicotiana tabacum*, plants by the tobacco mosaic virus increased resistance to green peach aphids, *Myzus persicae*. Direct and indirect interactions between other plant pathogenic fungi and insects species with either negative, positive or neutral outcomes for the insect are reviewed in Hatcher (1995).

The JA and SA pathways play an important role in plant defense against insect herbivores and pathogens. In the current study, we analyzed leaf tissue from peanut plants that were under individual or simultaneous attack by the white mold fungus and BAW to determine their levels of JA and SA. Peanut plants infected with the white mold fungus had levels of JA that were comparable to those induced by BAW damage alone. Additionally, plants that were infected with the fungus and exposed to BAW damage not only had significantly higher levels of JA than those damaged by either pest alone, but also had higher JA levels than could be explained by their additive effect upon the plant. JA was not detected in our control; it is possible that JA levels in healthy peanut plants fall below the range of sensitivity of the method used in this study. Since, SA has been reported to be involved in plant defense responses against pathogens, it was surprising to find that the levels of SA in peanut plants infected by *S. rolfsii* alone did not differ significantly from those of healthy plants. Because the levels of SA in white mold infected peanuts were actually lower than in healthy plants, it is unlikely that the feeding preference of BAW for white mold-infected peanut was because of SA suppression of JA-modulated defenses (Peña-Cortes et al. 1993; Doares et al. 1995). Furthermore, plants under synchronous attack by both pests had the highest levels of both JA and SA. Yet, it is interesting to note that despite the high levels of JA in these plants, the concentration of trypsin proteinase inhibitors, which might confer resistance to BAW (Broadway and Duffey 1986), were not different from those in all other plant treatments. Plants exposed to damage by BAW alone had the lowest levels of SA compared with all other treatments, so it appears that levels of this compound in peanuts are suppressed below normal levels when healthy plants are damaged by the insects alone.

It is now evident that the role of these two pathways in insect and pathogen defense is not as clear as previously thought. In fact, our data suggest that the JA rather than the SA pathway may be the one responsible for peanut responses against white mold infection, and that these pathways in peanut are up- or down-regulated depending on the number and type of biotic factors affecting the plant. The JA pathway has been previously reported to confer resistance in plants to other fungal pathogens (Vijayan et al. 1998 Cohen et al. 1993 Schweizer et al. 1993). Another mediator of plant defense responses is the highly volatile plant hormone, ethylene. Ethylene may interact with SA in inducing the expression of pathogenesis-related proteins involved in the induction of SAR. Ethylene, in conjunction with SA, is also believed to be responsible for the localized hypersensitive response of plants to pathogen attack by contributing to the development of necrotic local lesions that prevent the pathogen from spreading on to adjacent healthy tissues (re-

viewed in Chaudhry et al. 1998, O'Donnell et al. 2001). Jasmonic acid and ethylene also interact in the induction of a special kind of resistance, termed induced systemic resistance (ISR) (Chang and Shockey 1999, Ton et al. 2002). Thus, the potential role of ethylene in mediating and/or regulating insect and pathogen responses in plants needs to be investigated.

S. rolfsii is an opportunistic necrotrophic fungus, which, under favorable conditions eventually overcomes and kills its hosts. Thus, it is possible that the positive effect of its infection of peanuts on the performance of BAW may change with the amount of initial inoculum and also with the progression of the disease over time. For these reasons, it is important to note that the levels of fungal inocula used in our study were low and only caused a sub-lethal infection of the peanut plants.

Most studies conducted on the induction of plant defenses have focused on the effect of individual pest species; however, under natural field conditions, plants may often be under attack by different insect and pathogen pest species, or combinations thereof, at any given time. Understanding the innate mechanisms of defense in plants against multiple pests is vital for future development of resistance in crops. The data presented here contribute toward a better understanding of the effects of the interactions between insect and pathogen pests on their host plants. This type of information is important for the development of tools to prevent, manage, and control pests in agricultural environments.

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